The Association of Gastric Cancer Risk with Plasma Folate, Cobalamin, and *Methylenetetrahydrofolate Reductase* Polymorphisms in the European Prospective Investigation into Cancer and Nutrition

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Abstract

Previous studies have shown inconsistent associations of folate intake and polymorphisms of the *methylenetetrahydrofolate reductase* (*MTHFR*) gene with gastric cancer risk. Our nested case-control study within the European Prospective Investigation into Cancer and Nutrition cohort is the first prospective study of blood folate levels and gastric cancer. Gastric cancer cases (n = 247) and controls (n = 631) were matched for study center, age, sex, and time of blood donation. Two common single nucleotide

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polymorphisms of the *MTHFR* gene were determined, as were plasma concentrations of folate, cobalamin (vitamin B12), total homocysteine, and methylmalonic acid (cobalamin deficiency marker) in prediagnostic plasma. Risk measures were calculated with conditional logistic regression. Although no relations were observed between plasma folate or total homocysteine concentrations and gastric cancer, we observed a trend toward lower risk of gastric cancer with increasing cobalamin concentrations (odds ratio, 0.79 per SD increase in cobalamin; $P = 0.01$). Further analyses showed that the inverse association between cobalamin and gastric cancer was confined to cancer cases with low pepsinogen A levels (marker of severe chronic atrophic gastritis) at the time of blood sampling. The 677 C→T *MTHFR* polymorphism was not associated with gastric cancer, but we observed an increased risk with the variant genotype of the 1298 A→C polymorphism (odds ratio, 1.47 for CC versus AA; $P = 0.04$). In conclusion, we found no evidence of a role of folate in gastric cancer etiology. However, we observed increased gastric cancer risk at low cobalamin levels that was most likely due to compromised cobalamin status in atrophic gastritis preceding gastric cancer. (Cancer Epidemiol Biomarkers Prev 2007;16(11):2416–24)

Introduction

Gastric cancer is second only to lung cancer as a cause of worldwide cancer mortality (1). The estimated number of annual deaths worldwide is 850,000 (12% of all cancer deaths; ref. 1). The occurrence of gastric cancer has declined in industrialized countries over the past century but is still a major cause of cancer mortality in many developing countries (2). Although *Helicobacter pylori* (Hp) has been identified as a major cause of gastric cancer (3-5), diet and food preservation methods are still likely to modulate cancer occurrence (6, 7). The overall evidence from observational studies suggests a protective effect of, particularly, fruit, but also of vegetables (rich source of folate), on gastric cancer (6, 8). This evidence is weaker and not always statistically significant in recent meta-analyses of cohort studies (6, 8). Case-control studies with specific results on folate intake and gastric cancer risk suggest a protective role in some (9-13) but not all (14-16). A meta-analysis (17) showed a statistically nonsignificant weak protective effect of folate intake on gastric cancer risk among case-control studies, whereas two prospective studies did not observe any association (18, 19).

The evidence for a role of folate in cancer etiology has been strengthened by observations of a modulating role of the *methylenetetrahydrofolate reductase* (*MTHFR*) 677 C→T polymorphism in colorectal neoplasia (20, 21). The MTHFR enzyme activity, which is reduced in the TT genotype, affects folate distribution between separate folate species for nucleotide synthesis and DNA methylation (20, 22, 23). Notably, a series of case-control studies have shown that the variant TT genotype is a strong risk factor for gastric cancer in Chinese (24-28), Italian (29), and Mexican (30) populations but not in Korean (31), German (32), or Polish (33) populations. A meta-analysis recently summarized these studies and concluded that the relationship between *MTHFR* 677 C→T polymorphism and gastric cancer was present in East Asian but not in the other studied populations (34).

It is a weakness of previous studies on folate and gastric cancer that none were based on folate measurements in blood samples taken before the diagnosis of gastric cancer. Within the EurGast collaboration (35-39) of the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort (40, 41), we had the opportunity to conduct the first prospective study on gastric cancer risk and folate levels measured in blood. Additionally, we included measurements of plasma cobalamin, total homocysteine (tHcy), methylmalonic acid (MMA), and the two common *MTHFR* single nucleotide polymorphisms (SNP; 677 C→T and 1298 A→C) to provide a more comprehensive assessment of the role of B vitamins in gastric cancer.

Materials and Methods

Study Population and Collection of Blood Samples. The design and methods of the EPIC study have been previously described in detail (40, 42). Briefly, the EPIC cohort consists of 23 centers in 10 European countries (Denmark, France, Greece, Germany, Italy, the Netherlands, Norway, Spain, Sweden, and United Kingdom). Between 1992 and 1998, country-specific dietary questionnaires, standardized lifestyle and personal history questionnaires, anthropometric data, and blood samples were collected from most participants.

The present study includes gastric cancer cases who were diagnosed after blood collection and matched control subjects from all EPIC countries except Norway. In each of the recruitment centers, both fasting and nonfasting blood samples of at least 30 mL were drawn from all participants and stored at 5°C to 10°C protected from light and transported to local laboratories for processing and aliquoting as previously described (40, 42). The only exception is the EPIC-Oxford center where blood samples were collected from a network of general practitioners and transported to a central laboratory in Oxford via mail. Although protected from light, the whole blood samples were exposed to ambient temperatures for up to 48 h. As tHcy and folate measurements are compromised by such handling, EPIC-Oxford samples were excluded from the present analyses (three cases and nine controls).

In all countries, except Denmark and Sweden, blood was separated into 0.5 mL fractions (serum, plasma, red cells, and buffy coat for DNA extraction). Each fraction was placed into straws, which were heat sealed and stored at ultralow temperatures (−196°C) in liquid nitrogen. One half of all aliquots were stored at the local study center and the other half in the central EPIC biorepository at the IARC (Lyon, France). In Denmark, blood fraction aliquots of 1.0 mL were stored locally in Nunc tubes at −150°C under nitrogen vapor. In Sweden, samples were stored in −70°C freezers.

Follow-up for Cancer Incidence. In EPIC, follow-up is based on population cancer registries (Denmark, Italy,

the Netherlands, Norway, Spain, Sweden, and United Kingdom) and other methods, such as health insurance records, pathology registries, and active contact of study subjects or next of kin (France, Germany, and Greece). The follow-up period for the present study was for data reports received at IARC to the end of October 2002, representing complete follow-ups until either December 2000 or December 2001 for all centers using cancer registry data and until 2002 for France, Germany, and Greece. Cancers of the stomach included cancers coded as C16 (10th Revision of the International Statistical Classification of Diseases and Related Health Problems). The diagnosis, tumor site classification, and morphology (according to International Classification of Diseases for Oncology, second edition and Lauren classifications) of each identified cancer were confirmed and validated by an independent panel of pathologists with a representative from each EPIC country and a coordinator (43). The pathologist panel reviewed original histologic slides and/or recuts from the paraffin blocks and original histopathology reports that were provided by each EPIC center.

Nested Case-Control Study Design and Selection of Study Subjects. Incident gastric cancer cases were cohort members who developed cancer after recruitment into EPIC. The present study includes a total of 233 gastric adenocarcinomas and 14 adenocarcinomas of the gastroesophageal junction. These 247 cases are grouped together and referred to as gastric cancer. The cancer cases were divided into three groups by anatomic subsite: (*a*) tumors originating from the gastric cardia (n = 64 cases), combining tumors that reached the gastroesophageal junction, either crossing it or from below (all 14 gastroesophageal junction cancers) or not; (*b*) noncardial tumors (n = 112 cases), grouping cases from other sites in the stomach; and (*c*) tumors from unknown or mixed sites (n = 71 cases). When divided by histologic subtype, of the 247 gastric cancer cases, 85 were classified as diffuse and 85 as intestinal according to the Lauren classification. The remainder (n = 77 cases) were of unknown or mixed histologic types. All gastric lymphomas, gastric stump cancers, other gastric nonadenocarcinoma, esophageal nonadenocarcinomas, and otherwise unspecified malignant neoplasms of the stomach were excluded from this analysis. For each identified cancer case, control subjects with available blood samples were randomly selected from all cohort members who were alive and free of cancer (except nonmelanoma skin cancer) at the time of diagnosis of the case patient. A total of 631 controls were matched by gender, age group (±2.5 years), study center, and date of blood sample collection (±45 days). This study was approved by the Ethical Review Board of the IARC and those of all individual EPIC centers.

Laboratory Measurements. Plasma tHcy and MMA (marker for cobalamin status) were measured with a method based on methylchloroformate derivatization and gas chromatography-mass spectrometry (44). Plasma folate was determined by a *Lactobacillus casei* microbiological assay (45) and plasma vitamin B12 by a *Lactobacillus leichmannii* microbiological assay (46). Both the folate and vitamin B12 assays were adapted to a microtiter plate format and carried out by a robotic workstation (Microlab AT plus 2, Hamilton Bonaduz AG). The *MTHFR* 677 C→T (rs#1801133) and 1298 A→C (rs#1801131) polymorphisms were determined by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (47) in Bergen. In Barcelona, the same two polymorphisms were genotyped in a LightCycler 2.0 instrument (Roche Diagnostics) by real-time multiplex PCR and melting curve analysis of a fluorescently labeled sensor probe specific for each analyzed variant. Primers and probes (48) were synthesized by TibMolBiol. PCR mixtures and cycling conditions were essentially as described (49), with the exception that primers and probes for the two polymorphisms were mixed in the same reaction capillary (50).

When one of the Swedish centers was excluded because of small amount of DNA provided for matrix-assisted laser desorption/ionization time-of-flight analyses, the agreement between the Bergen and Barcelona laboratories were 99.7% to 99.9%. We gave preference to the genotyping results from the Barcelona laboratory in the three instances of discrepancies and for the Swedish center. Quantification of anti-Hp antibodies in stored plasma samples was done using an ELISA technique using incubation with lysates of the CCUG Hp strain (51). Pepsinogen A (PGA) was assayed in plasma by a commercial microplate-based quantitative ELISA kit (Biohit). Severe chronic atrophic gastritis was serologically defined as a PGA circulating level <22 μg/L (51).

We assessed correlations between estimated dietary folate and cobalamin intakes (mean levels were 268.3 and 7.3 μg/d) and the measured vitamin and marker levels among the controls. Spearman correlations (corrected for sex, age, energy intake, and country) between dietary folate and plasma folate and tHcy were 0.220 ($P < 0.0001$) and −0.124 ($P = 0.003$), respectively. Correlations between estimated intake of vitamin B12 and plasma cobalamin and MMA were 0.131 ($P = 0.002$) and −0.130 ($P = 0.002$), respectively.

Statistical Methods. Relative risks [odds ratios (OR)] and 95% confidence intervals (95% CI) for the gastric cancer cases and subgroups in relation to plasma concentrations of tHcy, folate, cobalamin, and MMA were calculated by conditional logistic regression using the SAS LOGISTIC procedure (SAS statistical software, version 9; SAS Institute), stratified by the case-control set. Risk estimates were computed with adjustments for total energy intake (in quartiles), Hp infection status, and smoking (variable categories: nonsmokers, ex-smokers, current smokers, and missing). These additional adjustments had only minor impact on the vitamin-gastric cancer associations. We also carried out analyses with additional adjustment for total carotenoids (38) and vitamin C (39).

The plasma analyte concentrations were examined by quartile categories with cut points based on the distribution of each specific analyte in all 631 controls combined. Models similar to the above were run with the logarithm of plasma measurements included in the model as continuous variables. In these models, the relative risk and 95% CI for each vitamin or marker were then calculated as the risk for a change in the plasma level by 1 SD. All the above models were run separately and tested for effect modification for each anatomic subsite (cardia, noncardia), histologic subtype (diffuse, intestinal), European region [northern-central, south (Italy, Greece, and Spain)], time from blood donation to cancer diagnosis (<2 years versus ≥2 years), age, sex, and Hp status.

The relationships between the two *MTHFR* polymorphisms and gastric cancer were studied with conditional logistic regression. The risk estimates were calculated with the wild-type as the reference category. A trend test with equally spaced integer weights for the genotypes was used to summarize the effect of each polymorphism. Additionally, we studied the two polymorphisms, which are in strong linkage disequilibrium ($D' = 0.999$, $r = -0.49$), using a haplotype approach. Our haplotype notation gives the 677-allele first and then the 1298 allele (e.g., c-a). Overall, both polymorphisms were in Hardy-Weinberg equilibrium ($P = 0.25$ for the *MTHFR* 677 C→T and $P = 0.61$ for the 1298 A→C polymorphism). Haplotype frequencies were estimated by assuming Hardy-Weinberg equilibrium and using the expectation-maximization algorithm (52). The t-c haplotype was extremely rare and therefore excluded from the analysis. Ignoring the t-c haplotype, the three remaining haplotypes could be reconstructed with certainty in both cases and controls. The three haplotypes (single or double copies) were used as exposures in a conditional logistic regression model. Because all individuals carry two haplotypes, one might see different types of interactions between the two haplotypes (i.e., recessive or dominant effects of one haplotype relative to another). To give a comprehensive presentation of effects, we used a ''reciprocal reference''; that is, we computed the effect of each specific haplotype relative to the remaining haplotypes, weighting according to the population haplotype frequencies (53). This means that, for any haplotype, the single-dose effect measures the relative increase (or decrease) in risk obtained by replacing any haplotype in an individual with this specific haplotype; the double-dose estimate is the effect of replacing both with this haplotype.

Effect modification of the folate-gastric cancer associations by the two *MTHFR* SNPs was studied with conditional logistic regression but stratifying on country instead of the matched sets and with age and sex as covariates.

Results

We studied 247 gastric cancer cases and 631 matched controls. Table 1 shows that the mean age at diagnosis was 62 years and that 40.5% of the cancer cases were women. Mean follow-up between blood donation and diagnosis of cancer was 3.3 years. Cancer cases had higher prevalence of positive Hp infection status and more smokers than their matched controls. Table 2 shows levels of folate, cobalamin, tHcy, and MMA among the 631 controls in categories by sex, age, smoking habits, European region, and the *MTHFR* polymorphisms. Whereas folate levels were lower in smokers ($P = 0.0005$), tHcy levels were higher in men ($P = 0.002$), in individuals >60 years of age ($P < 0.0001$), and in persons with the variant genotype (TT) of the 677 C→T polymorphism ($P = 0.002$). Cobalamin levels were higher in women ($P = 0.02$). MMA levels were lower in smokers ($P = 0.002$) and in controls <60 years of age ($P = 0.005$). Among the 247 cases, mean levels (SD) of the vitamins and markers were 13.9 (7.5) for folate, 10.7 (4.2) for tHcy, 284 (110) for cobalamin, and 0.203 (0.114) for MMA.

Table 1. Baseline characteristics and description of the study population of cases and controls

	Cases ($n = 247$)	Controls ($n = 631$)
Percent women	40.5	41.7
Age at recruitment, mean (min-max)	58.9 (43.3-70.3)	58.7 (42.7-71.4)
Age at diagnosis, mean (min-max)	62.2 (44.9-73.9)	NA
No. years from blood donation to diagnosis, mean (min-max)	3.3 (0.4-7.13)	NA
Percent Hp positive	84.9	67.3
Percent severe atrophic gastritis (PGA <22 μg/L)	18.1	8.0
Percent current smokers	29.6	22.0
Percent former smokers	36.0	33.1

Abbreviation: NA, not available.

B Vitamins and Gastric Cancer. We used quartiles as well as continuous representations of the B vitamin and marker levels to study associations with gastric cancer (Tables 3 and 4). For folate and tHcy, there was no evidence of associations with gastric cancer. For cobalamin, the conditional logistic regression analyses indicated a negative association with gastric cancer (OR, 0.79 per SD increase; $P = 0.01$). For MMA, the association with cancer was positive and weaker (OR, 1.17 per SD increase; $P = 0.06$). Further adjustment for total carotenoids or vitamin C levels did not change the results (data not shown).

We investigated the associations between cobalamin and MMA and gastric cancer further by analyzing separately cases with and without serologic evidence of severe atrophic gastritis (low levels of PGA). Table 3 shows that the associations between cobalamin and MMA and gastric cancer were confined to cases with low levels of pepsinogen. In this subgroup (44 cases), there were strong and highly significant dose-response relationships between gastric cancer and cobalamin (OR, 0.38 per SD increase; $P = 0.001$) and MMA (OR, 2.43 per SD increase; $P < 0.001$).

We further studied these cancer-metabolite associations in subgroups by anatomic subsite (cardia versus noncardia), geographic region, and histologic subtype (diffuse versus intestinal; Table 4). Folate showed opposite trends with respect to gastric cancer in cardia versus noncardia ($P_{\text{effect modification}} = 0.04$), but none of the subgroup effects were statistically significant. There was no effect modification of the cancer-metabolite associations by European region (northern-central versus southern Europe), sex, age (<60 or ≥60 years), or Hp infection status.

When we excluded the cancer cases with <2 years from blood sample to cancer diagnosis, the results were similar to the overall results given in Table 3.

***MTHFR* Polymorphisms and Gastric Cancer.** Table 5 shows that the homozygote variant genotype of the 677 C→T SNP of the *MTHFR* gene was more prevalent in southern Europe compared with central/northern Europe (21% versus 10% among controls), whereas no important difference in prevalence was seen for the CC genotype of the 1298 A→ C polymorphism (8% versus 9%). Although we observed no association between the 677 C→T polymorphism and gastric cancer, there was an

Table 2. B vitamin and marker levels [mean (SD)] in all control subjects (*n* = 631) by selected characteristics

	n	Folate (nmol/L)	tHcy (μmol/L)	Cobalamin (pmol/L)	MMA (μmol/L)
All controls	631	16.4 (12.9)	10.4 (3.3)	303 (170)	0.188 (0.096)
Sex					
Men	368	16.1 (13.4)	10.7 (3.1)	289 (175)	0.188 (0.084)
Women	263	16.9 (12.0)	9.9 (3.5)	322 (160)	0.188 (0.109)
P diff		0.45	0.002	0.02	0.97
Age (y)					
<60	305	16.3 (13.3)	9.7 (3.1)	305 (141)	0.177 (0.096)
≥60	326	16.6 (13.3)	11.0 (3.4)	301 (193)	0.198 (0.094)
P diff		0.77	<0.0001	0.77	0.005
Smoking					
Never	272	18.3 (14.8)	10.0 (3.1)	308 (154)	0.195 (0.109)
Former	209	16.1 (11.5)	10.6 (3.4)	290 (111)	0.194 (0.096)
Current	139	13.6 (10.5)	10.6 (3.7)	312 (257)	0.165 (0.06)
Unknown	11	12.2 (4.4)	10.9 (1.9)	294 (66.5)	0.171 (0.059)
P trend*		0.0005	0.14	0.82	0.002
Northern-central vs southern Europe					
Scandinavia, United Kingdom, and Central Europe	353	16.8 (14.5)	10.5 (3.2)	310 (192)	0.194 (0.090)
Italy, Spain, and Greece	278	15.9 (10.5)	10.1 (3.5)	294 (137)	0.180 (0.102)
P diff		0.33	0.16	0.24	0.07
MTHFR 677 C→T					
CC	248	18.1 (14.3)	10.2 (3.2)	296 (129)	0.188 (0.089)
CT	277	15.1 (8.7)	10.1 (3.0)	319 (214)	0.186 (0.091)
TT	94	16.3 (18.1)	11.4 (4.2)	277 (115)	0.191 (0.127)
P trend		0.26	0.002	0.38	0.78
MTHFR 1298 A→C					
AA	315	15.9 (12.3)	10.4 (3.4)	296 (133)	0.186 (0.095)
AC	246	16.8 (13.3)	10.4 (3.3)	315 (212)	0.186 (0.099)
CC	53	18.8 (14.7)	10.1 (3.4)	302 (164)	0.200 (0.081)
P trend		0.13	0.58	0.83	0.33

*Category unknown not included in trend test.

association of borderline significance (*P* for trend = 0.04) between the 1298 A→ C polymorphism and gastric cancer (OR, 1.47 comparing the CC genotype with the wild-type). Haplotype analysis confirmed that the risk increase was confined to the 1298 A→C polymorphism. Motivated by the higher prevalence of the 677 T allele in southern Europe, we did these analyses separately for European region, but we did not observe important differences in the cancer-SNP association.

Furthermore, we studied the association between plasma folate and gastric cancer in different genotypes of the two *MTHFR* SNPs. We found no evidence of effect modification of the folate-cancer association by the two SNPs (Table 6).

Discussion

In this large population-based European nested case-control study of gastric cancer, we found no evidence of a relationship between plasma folate and cancer but an inverse association of cobalamin to cancer. MMA (which

Table 3. ORs (95% CI) for gastric cancer (*n* = 245) by quartiles and per SD (log scale) of B vitamins and markers

	Q1*	Q2	Q3	Q4	*P* trend	Per SD (log scale) †	*P*
All cases							
Folate	1	1.03 (0.66-1.60)	0.83 (0.52-1.32)	0.92 (0.56-1.50)	0.56	0.90 (0.75-1.08)	0.27
tHcy	1	0.75 (0.47-1.21)	0.83 (0.52-1.33)	0.87 (0.52-1.44)	0.72	1.02 (0.85-1.21)	0.87
Cobalamin	1	0.91 (0.60-1.40)	0.60 (0.38-0.96)	0.66 (0.40-1.07)	0.03	0.79 (0.66-0.95)	0.01
MMA	1	0.90 (0.56-1.45)	0.81 (0.50-1.31)	1.35 (0.84-2.17)	0.22	1.17 (0.99-1.38)	0.06
Cases with low PGA (*n* = 44)							
Folate	1	1.90 (0.54-6.69)	1.41 (0.40-4.95)	1.45 (0.43-4.87)	0.70	0.86 (0.57-1.31)	0.49
tHcy	1	0.91 (0.30-2.79)	1.20 (0.41-3.48)	1.32 (0.42-4.11)	0.58	1.22 (0.81-1.85)	0.34
Cobalamin	1	0.18 (0.048-0.65)	0.21 (0.051-0.82)	0.080 (0.017-0.38)	0.002	0.38 (0.21-0.68)	0.001 ‡
MMA	1	2.28 (0.60-8.72)	1.98 (0.51-7.62)	4.29 (1.15-16.0)	0.04	2.43 (1.48-3.99)	<0.001 ‡
Cases with high PGA (*n* = 199)							
Folate	1	0.89 (0.55-1.44)	0.77 (0.46-1.29)	0.87 (0.50-1.52)	0.49	0.93 (0.75-1.15)	0.51
tHcy	1	0.68 (0.40-1.17)	0.79 (0.46-1.36)	0.80 (0.45-1.43)	0.66	0.98 (0.80-1.20)	0.85
Cobalamin	1	1.19 (0.74-1.92)	0.71 (0.42-1.20)	1.01 (0.59-1.74)	0.46	0.91 (0.75-1.12)	0.38 ‡
MMA	1	0.78 (0.46-1.31)	0.65 (0.38-1.11)	1.05 (0.62-1.78)	0.92	0.98 (0.81-1.18)	0.79 ‡

*Lower quartile is reference category. Adjusted for Hp status, smoking, and energy in conditional logistic model with stratification on matched sets.
† OR per SD (linear representation of vitamin/marker).
‡ Statistically significant different effect in cases with and without low PGA (<22 μg/L; *P* = 0.003 for cobalamin and *P* = 0.004 for MMA).

Table 4. ORs (95% CI) for gastric cancer for quartiles of B vitamins and markers by anatomic subsite, histologic subtype, and European region

	n cases/ *n* controls	Q1*	Q2	Q3	Q4	*P* trend	Per SD (log scale)†	*P* trend
Anatomic subsite								
Cardia	64/157							
Folate	64/155	1	1.96 (0.82-4.73)	1.73 (0.70-4.30)	2.01 (0.72-5.60)	0.23	1.26 (0.87-1.82)	0.22
Homocysteine	64/155	1	0.87 (0.33-2.27)	0.56 (0.22-1.43)	0.63 (0.22-1.87)	0.26	0.86 (0.57-1.29)	0.47
Cobalamin	64/155	1	0.90 (0.41-1.96)	0.60 (0.24-1.51)	0.64 (0.25-1.63)	0.23	0.81 (0.56-1.17)	0.26
MMA	64/155	1	0.49 (0.18-1.31)	0.74 (0.28-1.98)	1.27 (0.50-3.19)	0.31	1.29 (0.91-1.83)	0.15
Noncardia	112/285							
Folate	110/284	1	0.76 (0.35-1.62)	0.66 (0.31-1.40)	0.67 (0.32-1.44)	0.30	0.87 (0.66-1.14)	0.30
Homocysteine	110/284	1	0.86 (0.41-1.80)	0.90 (0.44-1.87)	0.72 (0.33-1.56)	0.46	0.87 (0.67-1.14)	0.31
Cobalamin	110/284	1	0.85 (0.41-1.73)	0.57 (0.26-1.21)	0.53 (0.24-1.17)	0.06	0.73 (0.55-0.96)	0.02
MMA	110/283	1	1.04 (0.51-2.13)	0.75 (0.36-1.58)	1.80 (0.86-3.77)	0.21	1.26 (0.97-1.64)	0.08
Histologic subtype								
Diffuse	85/226							
Folate	85/224	1	0.91 (0.43-1.93)	0.63 (0.27-1.45)	0.78 (0.34-1.82)	0.46	0.86 (0.63-1.16)	0.32
Homocysteine	85/224	1	0.49 (0.21-1.14)	0.65 (0.28-1.50)	1.00 (0.42-2.40)	0.78	1.11 (0.82-1.50)	0.52
Cobalamin	85/224	1	1.06 (0.49-2.28)	0.81 (0.37-1.78)	0.70 (0.29-1.69)	0.33	0.76 (0.55-1.04)	0.08
MMA	85/223	1	0.47 (0.20-1.09)	0.27 (0.11-0.69)	0.91 (0.40-2.06)	0.71	1.05 (0.80-1.37)	0.74
Intestinal	85/210							
Folate	84/208	1	0.89 (0.39-2.03)	1.12 (0.50-2.49)	0.79 (0.33-1.91)	0.77	0.90 (0.65-1.25)	0.52
Homocysteine	84/208	1	0.54 (0.24-1.22)	0.48 (0.22-1.05)	0.61 (0.26-1.46)	0.20	0.89 (0.65-1.22)	0.46
Cobalamin	84/208	1	0.93 (0.45-1.92)	0.40 (0.17-0.94)	0.57 (0.24-1.34)	0.06	0.81 (0.59-1.11)	0.19
MMA	84/208	1	1.50 (0.65-3.50)	1.63 (0.69-3.85)	2.61 (1.11-6.11)	0.03	1.43 (1.06-1.94)	0.02
Northern-central vs south‡								
Northern-central	154/353							
Folate	153/350	1	1.14 (0.64-2.03)	0.92 (0.49-1.73)	1.07 (0.57-2.02)	0.996	0.95 (0.76-1.20)	0.69
Homocysteine	153/350	1	1.39 (0.72-2.71)	1.06 (0.57-1.96)	1.00 (0.52-1.91)	0.69	0.99 (0.79-1.24)	0.90
Cobalamin	153/350	1	1.03 (0.59-1.82)	0.62 (0.33-1.14)	0.66 (0.34-1.25)	0.07	0.76 (0.59-0.97)	0.03
MMA	153/349	1	0.82 (0.43-1.55)	0.71 (0.37-1.37)	1.75 (0.94-3.26)	0.05	1.32 (1.06-1.64)	0.01
South	93/278							
Folate	92/276	1	0.84 (0.40-1.72)	0.71 (0.34-1.48)	0.69 (0.30-1.60)	0.34	0.80 (0.58-1.12)	0.20
Homocysteine	92/276	1	0.37 (0.18-0.79)	0.74 (0.33-1.65)	0.99 (0.41-2.37)	0.78	1.11 (0.83-1.47)	0.49
Cobalamin	92/276	1	0.72 (0.36-1.43)	0.48 (0.23-1.02)	0.54 (0.25-1.18)	0.06	0.76 (0.57-1.01)	0.06
MMA	92/275	1	0.99 (0.49-2.00)	0.94 (0.45-1.96)	0.90 (0.41-2.00)	0.77	1.03 (0.80-1.34)	0.81

NOTE: The only significant effect modification in the subgroup analyses was different folate-cancer associations in cardia versus noncardia ($P = 0.04$).
*Lower quartile is reference category. Adjusted for Hp status, smoking, and energy in conditional logistic model with stratification on matched sets.
† OR per SD (linear representation of vitamin/marker).
‡ Northern-central represents Scandinavia, United Kingdom, and Central Europe; south represents Italy, Spain, and Greece.

is elevated under cobalamin deficiency) showed a positive association with gastric cancer, whereas plasma tHcy was unrelated to cancer. Of the two common SNPs of the *MTHFR* gene, only the 1298 A→C SNP was associated with gastric cancer.

Strengths and Limitations. In contrast to previous prospective studies on folate and gastric cancer risk, we measured folate status in blood (plasma). A further strength of our study is the nested case-control design where cases are incident cases in a large multicountry cohort. Controls were matched to each case by age and sex and time and place of blood donation. In the EPIC study, extensive lifestyle and other relevant information and blood measurements have been collected on each individual. This facilitated comprehensive control of confounding and assessment of effect modification. All biochemical analyses were carried out in one laboratory and the presence of known relationships between tHcy and age, sex, smoking, and the 677 C→T *MTHFR* polymorphism (54) confirmed the data integrity of this multicenter study with local sample handling.

Gastric cancer is a complex disease with etiologic heterogeneity (55), and the number of cases in each anatomic subsite and histologic subtype was small and insufficient for precise estimation of risk measures in these subgroups. Nevertheless, with close to 250 cases, this is a large study. A short duration of follow-up, with a mean of ~3 years, between blood donation to cancer diagnosis may have biased the observed results. Experimental data suggest that folate may both prevent carcinogenesis and promote cancer growth, with the possible implication that it is folate status in early and young adult life that protects against cancer (56). Thus, it remains a possibility that vitamin status at younger ages may be a better predictor of later cancer, as progression to cancer through severe chronic atrophic gastritis (57) may disturb intestinal vitamin [particularly cobalamin (58)] absorption in the last year(s) before diagnosis. A null finding in the present study may represent the sum of two such opposite effects of folate. However, to disentangle such complex and time-dependent relationships is beyond the scope of our study and would require much longer follow-up and serial measurements of vitamins and its markers.

Although nutrient blood measurements escape methodologic problems with diet questionnaires and food composition tables, a limitation of the former is that blood levels may imperfectly reflect intake or total body stores of a nutrient and that handling of blood samples may affect measured nutrient levels.

Table 5. ORs (95% CI) for gastric cancer for the 677 C→T and 1298 A→C *MTHFR* SNPs and haplotypes by geographic grouping

	All countries			Scandinavia, United Kingdom, and Central Europe			Italy, Spain, and Greece		
	n cases/ *n* controls	OR (95% CI)	*P* trend	*n* cases/ *n* controls	OR (95% CI)	*P* trend	*n* cases/ *n* controls	OR (95% CI)	*P* trend
MTHFR 677	245/619			152/342			93/277		
CC	109/248	1		77/148	1		32/100	1	
CT	104/277	0.88 (0.63-1.23)		58/159	0.68 (0.44-1.03)		46/118	1.34 (0.78-2.32)	
TT	32/94	0.87 (0.54-1.40)	0.46	17/35	0.96 (0.50-1.79)	0.32	15/59	0.90 (0.44-1.87)	0.98
MTHFR 1298	244/614			151/342			93/272		
AA	103/315	1		64/169	1		39/146	1	
AC	116/246	1.37 (1.00-1.87)		72/141	1.32 (0.89-1.97)		44/105	1.46 (0.88-2.41)	
CC	25/53	1.47 (0.87-2.50)	0.04	15/32	1.30 (0.66-2.55)	0.20	10/21	1.81 (0.78-4.23)	0.08
Haplotypes*	244/612			151/340			93/272		
c-a single		0.73 (0.49-1.1)	0.12		0.83 (0.50-1.4)	0.48		0.64 (0.34-1.2)	0.17
c-a double		0.75 (0.44-1.3)	0.28		1.1 (0.55-2.0)	0.87		0.40 (0.15-0.99)	0.05
t-a single		0.87 (0.57-1.3)	0.52		0.67 (0.39-1.1)	0.13		1.4 (0.68-3.0)	0.35
t-a double		0.90 (0.53-1.5)	0.71		0.95 (0.48-1.9)	0.89		1.0 (0.42-2.3)	1.00
c-c single		1.6 (1.1-2.5)	0.02		1.9 (1.1-3.3)	0.02		1.2 (0.64-2.4)	0.53
c-c double		1.7 (0.94-3.1)	0.08		1.9 (0.85-4.0)	0.12		1.5 (0.61-3.8)	0.36

NOTE: Only cases and controls with B vitamin data included in the conditional logistic regression analyses with stratification on matched sets.
*Estimated haplotype frequencies are 0.34 for c-a, 0.37 for t-a, and 0.29 for c-c.

Interpretation. One motivation for studies of folate and gastric cancer has been the observed lower risk for such cancers in individuals with high intake of fruit and vegetables (6, 8). Folate is one of a multitude of potentially cancer chemopreventive compounds found in fruit and vegetables that could contribute to the protective effect. In a recent analysis of the EPIC-EurGast study (37), and also according to the results from other cohort studies (6, 8), no strong overall evidence was seen for a protective role of intake of fruit and vegetables in gastric cancer. The results from the EurGast study showed protective role of vegetable intake in the intestinal type of gastric cancer. Additionally, the EurGast analyses suggested a protective role of citrus fruit consumption in cardia gastric cancer (37). The lack of associations with prediagnostic plasma levels of folate and tHcy in the current nested case-control study is in line with these results and two prospective studies (17-19) on folate intake and gastric cancer risk that did not observe any association with dietary folate intake and gastric cancer.

We observed an inverse association between gastric cancer and cobalamin levels and a positive association with the cobalamin deficiency marker MMA. Taking the short follow-up into account, this finding could partly be due to impaired cobalamin status in atrophic gastritis (58) that often precedes gastric cancer (57). Our data support this hypothesis as the associations between cobalamin and its marker MMA and gastric cancer were confined to the cancer cases with serologic evidence of severe chronic atrophic gastritis.

The study of SNPs with metabolic effects has been used as arguments for a causal role of the affected metabolite in the disease under study (59). Both the 677 C→T and 1298 A→C SNPs of the *MTHFR* gene cause variant protein configurations and have metabolic effects on folate and tHcy levels, much stronger for the 677 C→T than for the 1298 A→C (despite the strong linkage disequilibrium close to 1; refs. 60, 61). The relationships of, particularly, the 677 C→T SNP with disease have been argued to imply causal roles for folate or homocysteine in disease etiology and progression (62, 63). Both the 677

Table 6. ORs (95% CI) for gastric cancer (*n* = 245) by plasma folate quartiles (Q1-Q4) and per SD (log scale) difference in folate by *MTHFR* genotypes

	Q1*	Q2	Q3	Q4	*P* trend	Per SD (log scale)†	*P*
Overall (245/626)	1	1.00 (0.65-1.53)	0.81 (0.51-1.28)	0.83 (0.52-1.34)	0.32	0.86 (0.72-1.03)	0.10
MTHFR 677 C→T							
CC (108/247)	1	1.29 (0.64-2.58)	0.85 (0.40-1.81)	0.93 (0.46-1.91)	0.61	0.83 (0.63-1.08)	0.16
CT (103/274)	1	0.94 (0.49-1.78)	0.82 (0.42-1.61)	0.63 (0.29-1.41)	0.26	0.85 (0.63-1.15)	0.29
TT (32/93)	1	1.10 (0.32-3.76)	0.51 (0.13-1.93)	1.33 (0.35-5.13)	0.88	0.90 (0.57-1.42)	0.64
P effect modification					0.83		1.00
MTHFR 1298 A→C							
AA (103/314)	1	0.82 (0.43-1.57)	0.55 (0.28-1.10)	0.90 (0.44-1.82)	0.47	0.86 (0.66-1.13)	0.29
AC (114/242)	1	1.01 (0.53-1.91)	0.91 (0.45-1.82)	0.75 (0.36-1.56)	0.43	0.86 (0.66-1.12)	0.25
CC (25/53)	1	1.10 (0.18-6.54)	2.70 (0.39-18.6)	1.04 (0.18-5.98)	0.90	0.71 (0.34-1.49)	0.37
P effect modification					0.79		0.69

*Lower quartile is reference category. Adjusted for age at recruitment, sex, Hp status, smoking, and energy in conditional logistic model with stratification on country (overall analyses adjusted for 677 and 1298 polymorphisms, respectively).
†OR per SD (log-linear representation of vitamin/marker).

C→T and the 1298 A→C SNPs have been extensively studied in case-control studies of pathologies as diverse as congenital malformations, cardiovascular disease, and many different cancers (20, 64). Generally, and also with respect to gastric cancer, most consistent associations with disease have been found for the 677 C→T SNP that also have more profound effects on plasma tHcy and folate levels (60). The number of controls in our study was too small to show the weaker relationships between the 1298 A→C polymorphism and folate and tHcy (60). A recent meta-analysis of the two *MTHFR* polymorphisms and gastric cancer showed that the T allele and TT genotype of the 677 SNP is a strong risk factor in East Asian (particularly Chinese) populations (34). No such relationship was found in European or other populations. The number of studies of gastric cancer and the 1298 A→C SNP was limited and allowed only to estimate its role in East Asian populations where it was observed to be a risk factor for gastric cancer. Thus, a null result for the 677 C→T SNP in our study of Europeans combined with a suggestion of an effect of the variant 1298 genotype on gastric cancer risk agrees well with the meta-analysis (34). Furthermore, we did not observe any evidence of effect modification by folate status on the *MTHFR*-cancer relationship as has been observed with colorectal cancer (63). Neither did additional analyses of cases originating from northern-central compared with southern Europe reveal any differential SNP-cancer relationships.

Conclusion. Although we found no evidence to support a role of folate in gastric cancer etiology, we observed increased gastric cancer risk at low cobalamin levels, most likely due to compromised cobalamin status in chronic atrophic gastritis that precedes gastric cancer. Of the two common SNPs of the *MTHFR* gene, the 1298 A→C variant genotype was associated with increased gastric cancer risk.

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